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Metabolic responses to starvation and feeding contribute to the invasiveness of an emerging pest insect, *Cacosceles newmannii* (Cerambycidae)

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Declarations

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Marion Javal: Conceptualization, methodology, writing – review & editing, supervision, project administration

Philipp Lehmann: Conceptualization, methodology, writing – review & editing, supervision

John S. Terblanche: Conceptualization, methodology, resources, writing – review & editing, supervision, project administration, funding acquisition

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Abstract

Metabolic rate, and the flexibility thereof, is a complex trait involving several inter-linked variables that can influence animal energetics, behavior, and ultimately fitness. Metabolic traits respond readily to ambient temperature variation, in some cases increasing relative or absolute energetic costs, while in other cases, depending on the organism's metabolic and behavioral responses to changing conditions, resulting in substantial energy savings. To gain insight into the rapid recent emergence of the indigenous South African longhorn beetle *Cacosceles newmannii* as a crop pest in sugarcane, a better understanding of its metabolic rate, feeding response, digestion times, and aerobic scope is required, in conjunction with behavioral responses to food availability or any limitation thereof. Here, we therefore experimentally determined metabolic rate, estimated indirectly as CO_2 production using flow-through respirometry, in starved, fasted, and fed *C. newmannii* larvae, at 20°C and 30°C. We estimated multiple parameters of metabolic rate (starved, standard, active, and maximum metabolic rates) as well as aerobic scope (AS), specific dynamic action (SDA), and the percentage time active during respirometry trials. Additionally, in individuals that showed cyclic or discontinuous gas exchange patterns, we compared rate, volume, and duration of cycles, and how these were influenced by variation in temperature. Standard and active metabolic rate, and AS and SDA were significantly higher in the larvae measured at 30°C than those measured at 20°C. By contrast, starved and maximum metabolic rates and percentage time active were unaffected by temperature. At rest and after digestion was complete, 35% of larvae showed cyclic gas exchange at both temperatures; 5% and 15% showed continuous gas exchange at 20°C and 30°C respectively, and 10% and 0% showed discontinuous gas exchange at 20°C and 30°C respectively. We propose that the ability of *C. newmannii* larvae to survive extended periods of resource limitation, combined with a rapid ability to process food upon securing resources, even at cooler conditions that would normally suppress digestion in tropical insects, may have contributed to their ability to feed on diverse low energy resources typical of their host plants, and become pests of, and thrive on, a high energy host plant like sugarcane.

Keywords: invasion biology, range expansion, metabolic flexibility, rate-temperature relationships, population dynamics, pest management

1. Introduction

Energy is a critical driver of animal life-history traits, like for example, growth and reproduction, with direct implications for population dynamics (Desforges et al., 2019). Insects, like all living organisms, actively acquire resources from their surrounding environments to meet their energetic requirements (Chown and Nicolson, 2004). Energy availability can set upper limits on population sizes, rates of proliferation or extinction and, ultimately, species richness of an area. Thus, areas with low energy availability result in smaller population sizes and greater extinction rates compared to areas with higher available energy (Carrara and Vazquez, 2010). This is known as 'species-energy theory' (Wright, 1983). The availability of energy is however not the only aspect that influences population dynamics. Indeed, the efficiency of resource acquisition, allocation, and utilization also plays an important role. The efficiency of energy use can be influenced by food quality, maturity or body size and transitions between hypo- or hyper-metabolic states such as quiescence, diapause, aestivation or reproductive dormancy (Storey, 2004) and by multiple environmental factors (e.g. photoperiod and temperature).

Measuring the efficiency of energy use is therefore of central importance for understanding species-energy theory, and studies of energy metabolism (specifically metabolic rates) provide insights into the cost of living in a particular environment and the costs of performing specific activities (e.g. locomotion, mating, growth) (Chown and Nicholson, 2004). One common approach to tackle questions about energy is to measure metabolic costs associated with different life-functions through respiratory metabolism, or metabolic rate. Metabolic rate is a complex trait with many parameters that can be readily extracted and compared for their functional relevance to animal biology. Among the most commonly investigated are aerobic scope (AS), which is the difference between standard metabolic rate (SMR) and maximum metabolic rate (MMR) (Wegener, 1996). Aerobic scope gives a measure of the potential scope for metabolic flexibility, and at least in fish, is thought to give an indication in the scope for growth, physiological regulation, fighting disease and coping with other stresses (Claireaux and Lefrancois, 2007; Clark et al., 2011; Pörtner et al., 2012; Steell et al., 2019). Aerobic scope is therefore viewed as a metric of an organisms' ability to cope with environmental demands (Claireaux and Lefrancois, 2007). Standard metabolic rate (SMR) is defined as the minimal maintenance metabolic rate of a post-absorptive resting ectotherm, below which physiological function is impaired, measured at a given temperature (Norin and

Malte, 2011). Within a particular species, variability in SMR can exist as a consequence of changing temperatures. Typically, insect's SMR are highly sensitive to acute warming, and cooling, with on average a doubling of SMR with a 10°C increase in ambient temperature, although there is much that can still be learned about the longer-term or evolutionary responses thereof (Irlich et al., 2009; reviewed in Chown and Nicolson, 2004).

An important metabolic parameter is the transient rise in metabolic rate following the ingestion of a meal (Karasov and Martinez del Rio, 2007; McCue, 2006; McCue, 2012).This process has been coined 'specific dynamic action' (SDA), or the 'heat increment of feeding' (HIF) (Nespolo et al., 2005), and has been observed in hundreds of animal species to date, including insects (Secor, 2009). This digestion-associated increase in metabolic rate following feeding reflects numerous processes involved in the breakdown, absorption and processing of food in the animal's gut (Karasov and Martinez del Rio, 2007). The SDA is a direct measure of energy acquisition efficiency and can represent a large component of an animal's energy budget (Steell et al., 2019). The SDA can be especially demanding under certain environmental conditions (McCue et al., 2016a; Rosendale et al., 2019). Several studies have reported a higher, but shorter, metabolic increase after ingestion at elevated temperatures, but the total energy allocated to SDA is usually considered as being independent of temperature (McCue et al., 2016a; Secor and Faulkner, 2002;Wang et al., 2003). In some cases, expressed as the cumulative costs, SDA declines as temperature rises (e.g. blood-feeding tsetse, McCue et al., 2016a; locusts, Miller et al., 2009) suggesting considerable energy saving can be affected by feeding and/or digesting at warmer temperatures. While feeding status, like temperature effects, is widely appreciated to increase SMR in insects (reviewed in Chown and Nicolson, 2004), only a handful of studies have tackled this question with sufficiently high temporal resolution to quantify the total or relative cumulative costs (see e.g. Table 1 in McCue et al., 2016a). Thus, whether SDA costs remain constant, or consistently change in any particular direction with temperature variation, is unclear for many taxa, including the important functional group of herbivorous insects.

Another important aspect of insect metabolism is the gas exchange pattern employed. At rest, the tracheated arthropods, including insects, exhibit at least three main types of gas exchange patterns namely, discontinuous, cyclic and continuous gas exchange (Chown and Nicolson, 2004; Matthews and Terblanche 2015). Gas exchange patterns vary between species, but also within a single species, depending on its physiological state, developmental stage, or environmental conditions, among other factors (Contreras and Bradley, 2010; Terblanche and

Woods, 2018, Javal et al., 2019). For insects that show discontinuous and/or cyclic gas exchange, a number of studies have investigated the acute effects of temperature (e.g. Terblanche et al., 2010). In some cases, the gas exchange patterns switch from being predominantly discontinuous to cyclic and/or continuous, or from cyclic to continuous, as temperatures increase (e.g. Contreras and Bradley, 2010; Thienel et al., 2015). Similarly, insects that exchange gases discontinuously switch to continuous gas exchange when food and water is provided (Duncan et al., 2002) although this may be driven to some extent by changes in feeding status and/or behavior. Nevertheless, the functional significance of different respiratory patterns employed by insects remain controversial and of broader evolutionary interest (Contreras and Bradley, 2010; Terblanche and Woods, 2018).

Food is a critical source of nutrients, but food shortages are commonly encountered, potentially leading to starvation stress (Zang et al., 2019). The duration of the food shortage determines the physiological mechanisms involved. Fasting is when an animal maintains physiological homeostasis while relying solely on endogenous nutrient reserves, and mainly occurs after short periods of food deprivation (Secor and Carey, 2016). Starvation, on the other hand, is when body mass is lost owing to depletion of these endogenous reserves after a longer period of food deprivation (McCue et al., 2017). Knowledge of physiological mechanisms to stressors such as starvation and temperature can help to reveal adaptive strategies and predict the ability of insect pests to thrive in novel environments. Insects that employ these physiological adaptations can increase survival probability under abiotic stress (such as extremes of temperature and starvation). For example, they can maximise the amount of resources stored that are consumed during stress or they can utilize resources conservatively by reducing the rates at which they are consumed (Gibbs et al., 2003; Marron et al., 2003; Matzkin et al., 2009). The latter can be achieved by reductions in metabolic rate, partly driven by behavioural responses such as quiescence (Storey, 1998; Marron et al., 2003). For example, heat induced suppressions of metabolic rate has been found in marine snails, which improves their energy conservation in high-temperature environments (Marshall and McQuaid, 2011).

Cacosceles newmannii (Thomson 1877) (Coleoptera: Cerambycidae) is a longhorn beetle that was found feeding on sugarcane for the first time in 2015 in the KwaZulu-Natal province of South Africa, where its larvae cause significant damage to sugarcane stalks (Way et al., 2017). Records show that this species has historically been recorded in diverse locations within South Africa, as well as in Mozambique and Eswatini, and scant information is

154 available regarding its primary host plants (Ferreira, 1980; Smit et al., in review).
155 Considering the potential of *C. newmannii* to spread and cause significant agricultural losses,
156 having the ability to predict its metabolism and related traits under diverse conditions is
157 critical. Here we focus on the larval life stage because, to our knowledge, *C. newmannii*
158 adults do not feed (Way et al., 2017). There has been a long interest in the biology of
159 nutrition, starvation and energy use in Cerambycidae larvae. The group is generally well
160 known for having long-life cycles, consuming relatively low energy host plants or parts of
161 plants, for example dead or decaying wood, a tissue especially poor in nitrogen and therefore
162 a low source of amino acids and nutrients (Hanks, 1999; Mattson, 1980), and being generally
163 resilient to environmental stress (Ivanovic et al., 2002; Munyiri and Ishikawa, 2005;
164 Walczynska, 2007, 2009). However, few studies to date have employed high resolution flow-
165 through respirometry under estimated diverse feeding states at controlled temperatures to
166 quantify key digestion-related metrics (such as SDA). Furthermore, while adults have had
167 their gas exchange patterns and associated metabolic characteristics assessed (e.g. Chappell
168 and Rogowitz, 2000), larvae have, to our knowledge, not received similar attention. Our
169 earlier work on *C. newmannii* showed switching between discontinuous and continuous gas
170 exchange patterns in adults and larvae under experimentally manipulated oxygen levels
171 (Javal et al., 2019), but gas exchange characteristics have not yet been thoroughly explored
172 and reported. We hypothesized that *C. newmannii* has physiological characteristics of
173 respiratory metabolism that might explain its abrupt occurrence as a pest in sugarcane. For
174 example, larvae could potentially face resource limitations and starving if between host
175 plants, or if fields are harvested, or when they move to the soil before pupating.

176 The main aim of this study was to gain a better understanding of the rapid, unexpected
177 emergence of *C. newmannii* as a pest in sugarcane. The specific objectives for this study were
178 therefore to determine: (i) what the relative ability of *C. newmannii* larvae is to survive under
179 conditions of starvation and desiccation, (ii) what the effects of controlled, ecologically-
180 relevant temperature variation on diverse metabolic parameters are, including starved
181 metabolic rates, standard metabolic rates (SMR), active metabolic rates (AMR), maximum
182 metabolic rates (MMR), aerobic scope (AS), and estimate specific dynamic action (SDA)
183 and, percentage time active, (iii) the extent of possible metabolic suppression that could help
184 *C. newmannii* larvae cope during resource-poor conditions by comparing starved metabolic
185 rates to SMR at 20°C and 30°C, and finally, (iv) the responses of gas exchange patterns by

characterizing pattern, burst rate, volume and duration, and interburst rate, volume and duration, at these controlled temperatures.

2. Materials and methods

Animals

Small- and medium-sized (ranging from 0.091 to 4.64 g) *Cacosceles newmannii* larvae were collected by hand on sugarcane farms in the Entumeni area (28°55'S; 31°19'E) of KwaZulu-Natal. Larvae were primarily found in the base of sugarcane stalks that had to be split open to collect the larvae inside. Larvae were taken to the laboratory at the South African Sugarcane Research Institute (SASRI) in KwaZulu-Natal, South Africa and kept in moist peat (to provide sufficient food and water) at 23 °C in a 16 L: 8 D regime. The larvae were then couriered overnight, individually placed in plastic 250 ml jars filled with peat, to the Department of Conservation Ecology and Entomology, Stellenbosch University, Western Cape Province, South Africa where the experiments described below took place.

Experiment one: Survival assay

A survival assay was conducted on 51 larvae for 45 days by subjecting individuals to different treatments and monitoring their survival every day. The different treatments were a starvation (n=17), a desiccation (n=17), and a control (n=17) treatment. For all three treatments the larvae were removed from peat and placed in individual containers (200 ml). For the starvation treatment, the individual containers were placed in a bigger container with distilled water. Each individual container had moist cotton wool in (to provide water to larvae) and no food. For the desiccation treatment individual containers, without food and water, were placed in a bigger air-tight container half-filled with silica gel. Individual containers for the control treatment contained a 10-15cm piece of sugarcane and moist cotton wool for water and they were placed in a bigger container with distilled water. Temperature (°C) and relative humidity (%) were recorded for all 3 treatments using hygrochron iButtons (Maxim ibutton, Hygrochron Hi-Res (-20 °C to +85°C) Acc 0.5°C, USA) set to two-minute sampling frequencies. For the desiccation treatment, the average temperature recorded over 45 days was 29.2°C and relative humidity was recorded as 17%. For the starvation treatment, the average temperature recorded over the 45 days was 29.7 °C and relative humidity was 100%. The control treatment experienced an average temperature of 29.8 °C and relative

humidity of 102%. The experiment was terminated at 45 days owing to equipment constraints.

Experiment two: Respirometry of unfed larvae

Twenty-three new larvae were removed from the peat and kept individually in 250 ml jars containing only wet cotton wool, in a 16 L: 8 D regime, without food for between five and nine days. Preliminary experiments showed the cessation of excretion of faeces, indicating that larvae were no longer digesting food, a maximum of three days after they were removed from peat. Therefore, any period longer than three days was considered the transition from a 'fasting' state to 'starvation' state. Following the period without food, the larvae were weighed and randomly placed at either 20°C (n=10) or 30°C (n=13) and metabolic rate was measured using flow-through respirometry as detailed below. The temperatures were chosen because they are within the thermobiological range likely to be experienced in the field by the species (Javal et al., 2018; Kleynhans et al., 2018; SASRI, 2020). Additionally, profound differences in metabolic rates and gas exchange pattern might be expected across a 10°C range, given responses of other species reported to date (e.g. Basson and Terblanche, 2011; Chown et al., 2006), and as such can give insight into the thermal sensitivity of the traits considered here.

Experiment three: Respirometry of fed larvae

After a starvation period of between five to nine days (as described above), larvae were given the opportunity to feed on sugarcane, at room temperature, by placing them individually inside sugarcane stalks, approximately 15 cm in length, and checking them daily. When feeding damage could be visually observed on the sugarcane, larvae were weighed. Larvae were considered as fed as soon as they gained more than 2% of their initial body mass. For each larva, the percentage increase in body mass was calculated and used as an indication of meal size. They were then randomly placed at either 20°C (n=10) or 30°C (n=10) inside flow-through chambers and their metabolic rates were estimated as described in detail below. Metabolic rates were estimated continuously for >15 hours in order to fully characterize the metabolic response after a meal (Secor, 2009).

Metabolic rates

Metabolic rates were estimated indirectly as carbon dioxide (CO_2) production using flow-through respirometry. For the respirometry of unfed and fed larvae (experiment two and

three), they were randomly placed at either 20 °C or 30 °C, maintained using a circulating, programmable refrigeration liquid bath (CC-410wl, Huber, Berching, Germany), inside flow-through chambers (20 or 30 ml syringes depending on animal size). Individuals were given approximately ten minutes to settle in the chambers before starting the recording. Air, scrubbed of CO_2 and water vapour, was flowed through each chamber containing an animal for a metabolic rate estimation following methods adapted from e.g. Boardman and Terblanche (2015).

Briefly, metabolic rate was estimated by measuring the rates of carbon dioxide production (VCO_2), in ppm, using a calibrated Li-Cor infrared CO_2 analyzer with Li-Cor software (LI-7000 Windows Software Version 2.0.0). Flowrate was regulated to 200 ml min^{-1} STPD by a calibrated flow control valve (model no. 840L-2-OV1-SV1-E-V1-S1, Sidetrak, Sierra International, USA). During the measurement, activity was monitored using infrared scatter-laser-diodes (Sable Systems AD-1 activity detector) connected to a desktop computer to record and quantify movement of the animals, sampled at one Hz. This ensured that sections of the recording showing increased CO_2 production associated with activity could be readily detected (Fig. 1). A few individuals were recorded without an activity detector, depending on availability of the AD-1 activity detector equipment. Baseline recordings of about ten minutes were taken before and after each respirometry run to correct for potential analyzer drift. Mass was recorded before and after each respirometry recording using a calibrated electronic balance (to 0.1mg, Mettler Toledo MS104S/01, Switzerland). The mean mass for the respirometry recording was used in all further calculations. Respirometry files were converted from ppm to mL CO_2 per hour and extracted using Expedata (version 1.9.10, Sable Systems), after correction for baseline drift, even though this was generally non-existent. The Li-Cor infrared CO_2 analyzer simultaneously measured H_2O concentrations but for the purpose of this study the H_2O data was disregarded. Two identical respirometry systems were used in parallel (i.e. two separate Li-Cor infrared CO_2 analyzers were used) in order to measure more than one individual at the same time. Since the survival assay (experiment one) had the potential to cause stress and extensive use of energy reserves for the larvae, the larvae used for this experiment were not used for any other measurements. However, eight larvae used for respirometry of unfed larvae (experiment two) were also used for respirometry of fed larvae (experiment three). In addition, 15 larvae were only used for respirometry of unfed larvae (experiment two) and 12 larvae only for respirometry of fed larvae (experiment three).

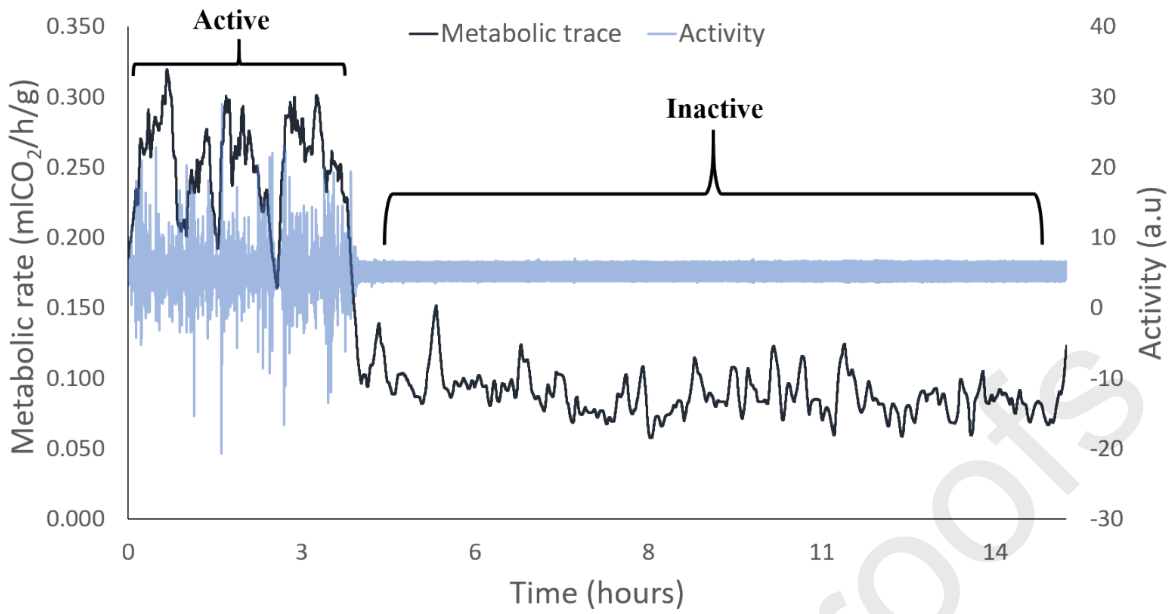


Fig. 1. An example of a metabolic rate (respirometry) recording in a typical experiment, estimated from CO_2 production, of a *C. newmannii* larva illustrating how increased CO_2 production associated with activity were identified. Activity is shown in a. u. Larva mass= 2.7694 g, flow rate= 200 ml min^{-1}, temperature= 30°C.

- *Starved metabolic rates*

The respirometry recordings of larvae that were starved for between five and nine days were used to determine starved metabolic rates. The activity data in these recordings were used to identify metabolic traces representative of non-active, stable metabolic rates of larvae. In some cases, we recorded individuals without an activity detector owing to equipment constraints. Therefore, we used those individuals with both activity and VCO_2 recordings to estimate typical metabolic rate variability and assumed similarly in individuals when only VCO_2 was recorded. The starved metabolic rates were then calculated from the lowest average of continuous CO_2 production recorded for 40 minutes of metabolic traces where larvae are inactive at 20°C (n=10) or 30°C (n=13). A period of 40 minutes was chosen for calculations of starved, standard, active and maximum metabolic rates because this amount of time was sufficient to include at least one cycle in their respiratory pattern. Starved metabolic rate thus represents the metabolic rate of larvae that are inactive and under starvation stress.

- *Standard metabolic rates (SMR)*:

The respirometry recordings of larvae that fed on sugarcane and gained more than 2% of their body mass were used to determine SMR, AMR, MMR, AS, SDA, and proportion spent

active. Activity data was used to identify metabolic traces representative of stable, resting metabolic rate of inactive individuals that are no longer digesting. Similar traces were then selected for individuals that activity was not recorded for. Standard metabolic rate (SMR) was calculated from the lowest average of continuous CO_2 production recorded for 40 minutes of respirometry traces where larvae are inactive at 20 °C (n=10) or 30 °C (n=10) (Fig. 2). SMR thus represents the metabolic rate of larvae that are inactive and post absorptive but not yet likely to be starving.

- *Active metabolic rates (AMR)*

Activity traces were used to identify periods of increased CO_2 production associated with activity, representative of active metabolic rates. Recordings of individuals that activity was not recorded for or individuals that were inactive the whole time they were in a postabsorptive state, were not used. Active metabolic rates (AMR) were calculated from the highest average of continuous CO_2 production recorded for 40 minutes of selected traces of larvae 20°C (n=4) or 30°C (n=3) (Fig. 2). AMR thus represents the metabolic rate of larvae that are active and post absorptive but not yet likely to be starving.

- *Maximum metabolic rates (MMR)*

Within the first few hours of the recordings of larvae that fed, we could not disentangle activity and digestion related increases in metabolic rate. We therefore calculated MMR from the highest average of continuous CO_2 production recorded for 40 minutes within the first few hours of recordings of larvae at 20°C (n=10) or 30°C (n=10) (Fig. 2). MMR thus represents the metabolic rate of larvae that are active and digesting.

- *Aerobic scope (AS)*

Aerobic scope was calculated as the difference between MMR ($mlCO_2/h/g$) and SMR ($mlCO_2/h/g$) of larvae at 20°C (n=10) or 30°C (n=10) (Fig. 2). Therefore, this can be considered the absolute aerobic scope (Halsey et al., 2018).

- *Specific dynamic action (SDA)*

SDA was calculated by first plotting moving average of 600 seconds of VCO_2 data against time (hours) and adding a threshold line representing a baseline metabolic rate (Fig. 2). Thereafter SDA ($mlCO_2/g$) was calculated from the extra VCO_2 above the baseline metabolic rate over the duration of digestion. SDA was therefore estimated as the CO_2 volume produced

during digestion subtracted from the baseline metabolic rate cost. The baseline metabolic rate was calculated from the lowest average of continuous CO_2 production recorded for 240 minutes of metabolic traces where larvae are inactive at 20°C (n=10) or 30°C (n=10). Digestion was considered to be complete when the respirometry trace reached a point on the graph below this threshold (Fig. 2). Many larvae breathed cyclically (showed a pattern of spikes and dips in their metabolic traces) and moving average data was used in order to better identify patterns in the data.

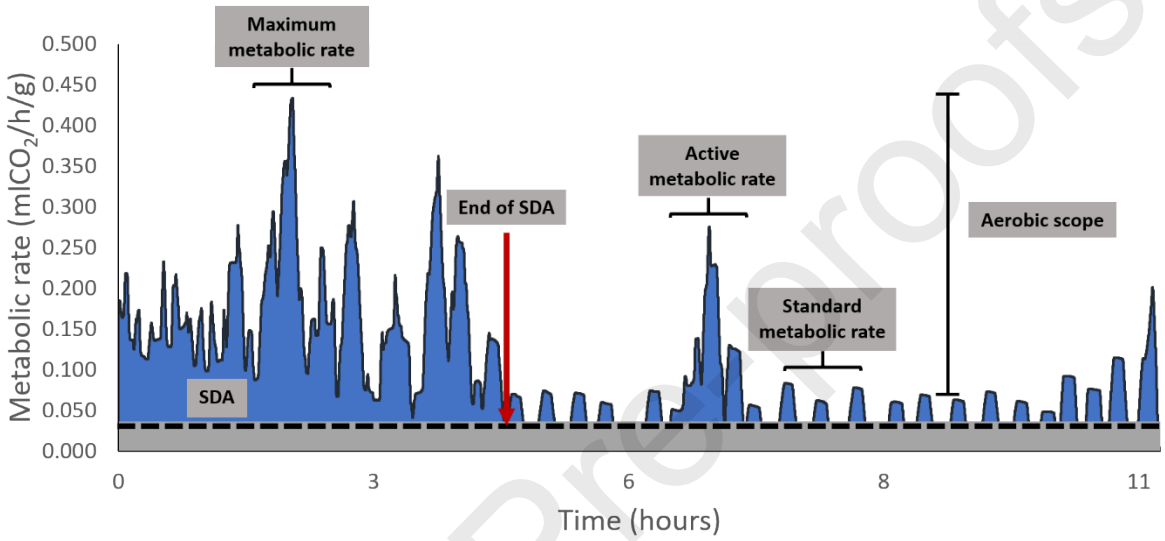


Fig. 2. An example trace (of moving average data of 600 seconds, sample frequency 1Hz) showing how SDA (specific dynamic action) was determined. Metabolic rate (in $mlCO_2/h/g$) is shown plotted against time (hours) for an individual larva that ate sugarcane (meal size: 4 % of body mass, initial mass: 1.0697 g) showing standard metabolic rate calculated as the average of the lowest continuous 40 minutes when larvae are post absorptive, maximum metabolic rate, active metabolic rate and specific dynamic action (SDA). The red arrow shows the time at which the respirometry trace reached a point on the graph equal or below the black line representing a baseline metabolic rate (calculated as the average of the lowest continuous 240 minutes when larvae are post absorptive), thus representing the end of SDA (specific dynamic action). Aerobic scope (calculated as difference between maximum metabolic rate and standard metabolic rate) is also illustrated on the graph.

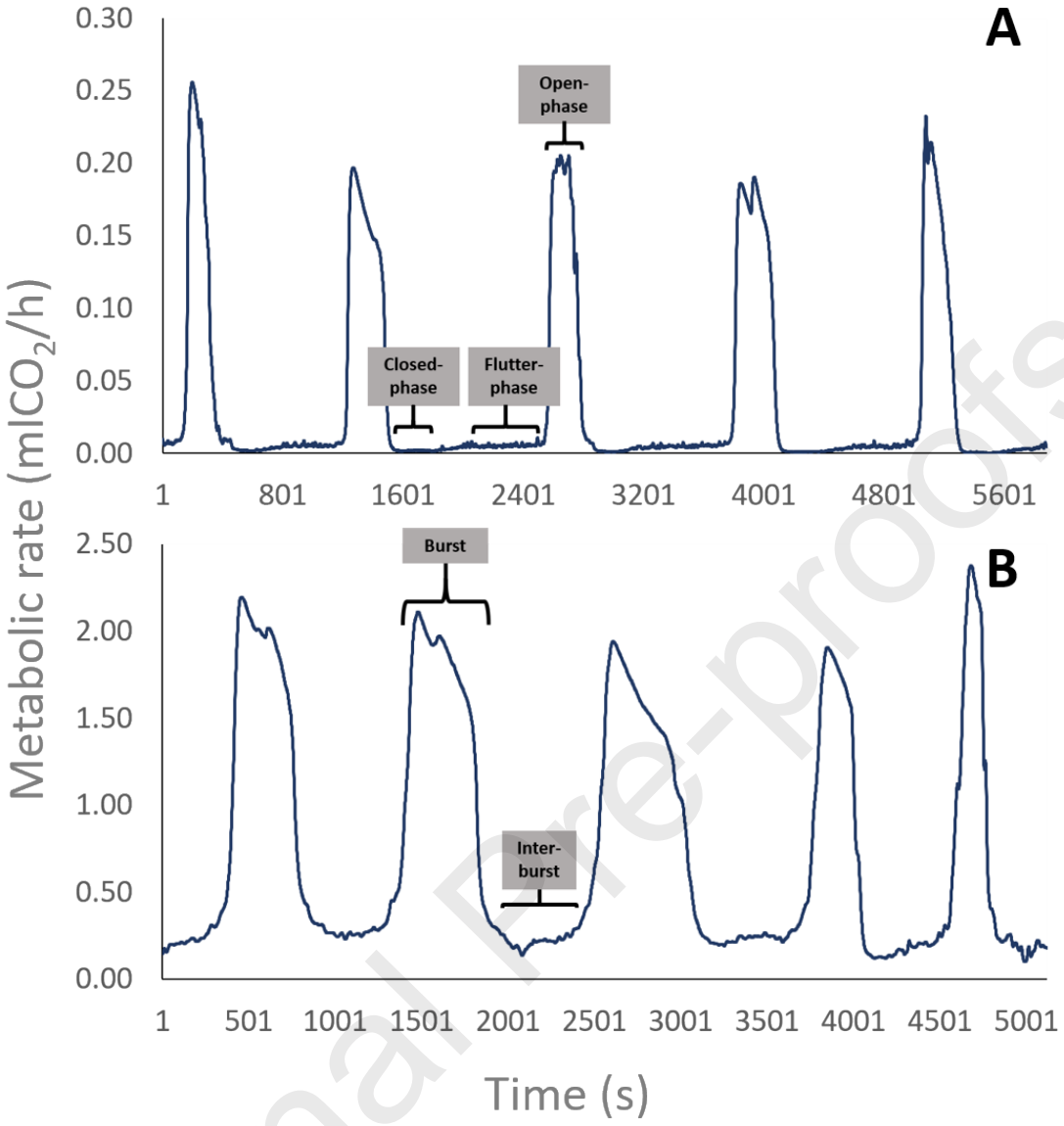
- *Percentage time active (%)*

For the larvae that fed and also had their activity measured (n=12) we determined the amount of time (in seconds) the larvae spent active within the first 14 hours (50 400 seconds) of the

recordings in order to calculate the percentage activity time of each individual larva at 20°C (n=5) or 30°C (n=7).

- *Gas exchange characteristics*

Using inactive periods of postabsorptive larvae, we determined the gas exchange pattern type (continuous, cyclic or discontinuous) first by inspection, and then according to the method proposed by Marais et al. (2005): from the raw respirometry trace ($mlCO_2/h$), the gas exchange pattern was named cyclic or discontinuous gas exchange when less than 30% of data points lay above the average VCO_2 line, then discontinuous gas exchange is readily identified due to the presence of a true closed (C) phase (i.e. the VCO_2 trace goes down to zero) (Fig. 3). When more than 30% of data points are above the average line, the gas exchange pattern labelled was continuous gas exchange. We calculated the mean VCO_2 from the average of the maximum number of continuous cycles (ranging from 3 to 34 cycles) at rest in individual recordings. For individuals that showed cyclic gas exchange we calculated burst rate ($mlCO_2/h$), burst volume ($mlCO_2$), burst duration (s), interburst rate ($mlCO_2/h$), interburst volume ($mlCO_2$), and interburst duration (s). The interburst period was characterized as the period between the lowest value at the end of a burst and the corresponding value preceding the following burst. Burst periods were those periods between interburst periods (Chown et al., 2006) (Fig. 3). For individuals that showed discontinuous gas exchange we calculated rate ($mlCO_2/h$), volume ($mlCO_2$), and duration (s) for the open (O-) phase and rate ($mlCO_2/h$), volume ($mlCO_2$), and duration (s) for the closed-flutter (CF-) phase. The closed and flutter phases were combined since it was not possible to easily distinguish the transition between these two phases (Fig. 3). For each individual, three cycles were used to calculate the respective rates ($mlCO_2/h$), volumes ($mlCO_2$) and durations (s) at 20°C (n=7) or 30°C (n=7) and the means of these values formed the individual data that were used for subsequent statistical analyses.



377

378 **Fig. 3.** An example of a metabolic rate (flow-through respirometry) recording in a typical
379 experiment, estimated from CO_2 production, of *C. newmannii* larvae measured at 20°C
380 illustrating the difference between (A) discontinuous gas exchange characterized by closed- ,
381 flutter- and open-phase and (B) cyclic gas exchange divided into burst and interburst periods.
382 Mass of animal in A: 1.0309g, and B: 3.3881g, flow rate=200 ml min^{-1}, sampling rate=1Hz.

383 *Statistical analysis*

384 Key assumptions for statistical tests were checked and not violated in the results presented.
385 Statistica 13 (TIBCO Software, United States) software was used to perform all statistical
386 tests except the survival analysis, which was done using R studio (R version 4.0.0, R core
387 team, 2018). Differences were accepted as statistically significant at a 5% probability level.

Experiment one: Survival assay

To determine if there was a difference in the survival of larvae between different treatments (desiccation, starvation, and control), we did a survival analysis comparing multiple groups. We used a Cox Proportional Hazards Regression analysis to determine if there is a significant difference in the mortality of larvae in the different treatment groups over the 45 days they were monitored.

Experiment two: Respirometry of unfed larvae

Body mass (in g) between starved larvae measured at 20 °C and 30 °C was compared using a t-test for independent samples. To determine if we should correct for mass, we compared the slope of the relationship between body mass and starved metabolic rate ($mlCO_2/h$) to a slope value of 1 using a one sample t-test. A t-test for independent samples was used to compare mass-adjusted starved metabolic rates ($mlCO_2/h/g$) between 20°C and 30°C degrees. To determine if there is an association between the starved metabolic rate ($mlCO_2/h/g$) and the amount of days the larvae were starved, a correlation was done between log transformed starved metabolic rates and days starved.

Experiment three: Respirometry of fed larvae

Body mass (in g) between fed larvae measured at 20°C and 30°C was compared using a t-test for independent samples. In order to determine if there was a linear relationship between the body mass (g) and SMR ($mlCO_2/h$) of larvae we compared the slope of the relationship between log transformed data of body mass (g) and SMR ($mlCO_2/h$) to a slope value of 1 using a one sample t-test. Similarly, to determine if there was a linear relationship between the body mass (g) and AMR ($mlCO_2/h$) of larvae we compared the slope of the relationship between the two variables (log transformed) to a slope value of 1 using a one sample t-test. The same was done for MMR ($mlCO_2/h$). Subsequently, the mass adjusted SMR and MMR ($mlCO_2/h/g$) of larvae measured at 20°C and 30°C were compared using a nonparametric Mann-Whitney test. The mass adjusted AMR (mlCO2/h/g) was compared using a t-test for independent samples. AS ($mlCO_2/h/g$), SDA ($mlCO_2/g$), and proportion spent active (%), between larvae measured at 20°C and 30°C, was also compared using a nonparametric Mann-Whitney test.

For the larvae that was used for both respirometry of unfed and fed (experiment two and three), we managed to measure the starved metabolic rate and SMR of the same individual.

For these individuals, their starved and standard metabolic rates ($mlCO_2/h/g$) were compared at 20°C (n=4) and 30°C (n=4) using a Wilcoxon matched pairs test. To determine if there is an association between MMR ($mlCO_2/h/g$) and meal size (% initial body mass), a standard Pearson correlation was performed between log transformed MMR ($mlCO_2/h/g$) and meal size (% initial body mass). To determine if there is an association between SDA ($mlCO_2/g$) and meal size (% initial body mass), a Pearson correlation was undertaken between log transformed SDA ($mlCO_2/g$) and meal size (% initial body mass). For the larvae that showed cyclic gas exchange, the burst rate ($mlCO_2/h$), volume (ml CO_2), and duration (s) as well as the interburst rate ($mlCO_2/h$), volume ($mlCO_2$), and duration (s) at 20°C and 30°C was all separately compared using a t-test for independent samples.

3. Results

Experiment one: Survival assay

The results of the survival analysis showed no significant difference in the survival of the larvae from the three treatment groups (χ^2=1.44, df=2, p=0.5) (Fig. 4). Furthermore, the survival assay revealed that larvae are tolerant of resource limitations since, even after 45 days, more than 70% of the larvae were still alive regardless of the treatment conditions.

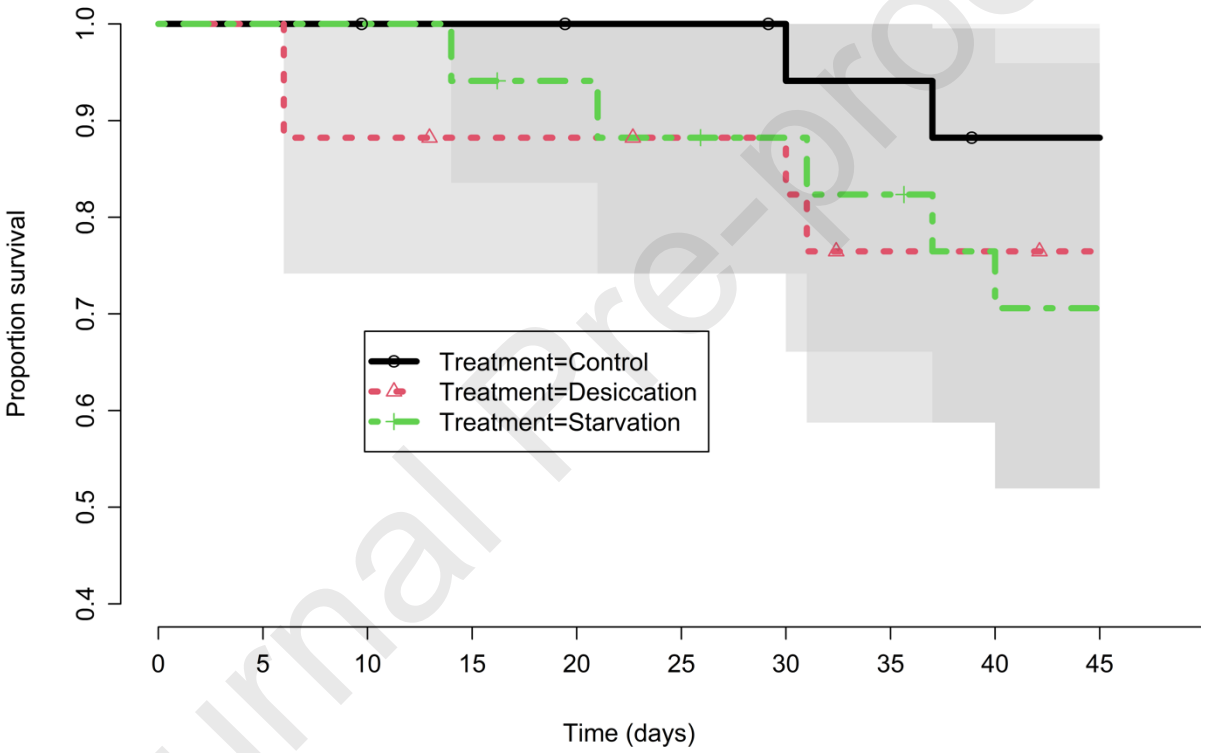


Fig. 4. Survival plot showing proportion survival (with 95% confidence intervals shown in grey) of *C. newmannii* larvae under desiccation, starvation and control treatments for 45 days.

Experiment two and three:

- *Temperature effects on starved metabolic rates*

We analysed the data of the starved metabolic rates of n=10 larvae measured at 20°C and n=13 larvae measured at 30°C (Supplementary material: Table S1). The average mass (g) of larvae (calculated as the average of mass before and after a respirometry recording) measured

at 20°C did not significantly differ from the average body mass (g) of larvae measured at 30°C (Table 1). There was no significant difference between the slope of body mass (g) and starved metabolic rates ($mlCO_2/h$) of larvae compared to a line of equivalence, i.e. 1.0 (t_{21}=0.344, p=0.367). These results suggested that there is a linear relationship between the body mass and starved metabolic rates of larvae. We therefore decided to do further analyses with mass-adjusted starved metabolic rate ($mlCO_2/h/g$) data. The results showed that starved metabolic rate ($mlCO_2/h/g$) of larvae measured at 20°C and 30°C did not differ significantly (Table 1). There was no correlation between starved metabolic rates ($mlCO_2/h/g$) and duration of starvation at 20°C (r=0.300, p=0.399) or at 30°C (r=0.350, p=0.241).

- *Temperature effects on metabolic parameters and behaviour*

The average mass (g) of larvae (calculated as the average of mass before and after respirometry recording) measured at 20°C was not significantly different compared to the average body mass (g) of larvae measured at 30°C (Table 1). There was no significant difference between the slope of the relationship of SMR ($mlCO_2/h$), MMR($mlCO_2/h$) or AMR ($mlCO_2/h$) and body mass (g) and a line of hypothetical equivalence, i.e. 1.0 (t_{18}=0.151, p=0.559; t_{18}=-0.223, p=0.413; t_5=-0.294, p=0.390 respectively) suggesting that there is a linear relationship between body mass (g) and SMR, MMR, and AMR of larvae. We therefore decided to do further comparisons using mass-adjusted data (Supplementary material: Fig. S1; Table S2). Results of comparisons between 20°C and 30°C of mass-adjusted SMR, AMR, MMR, AS, SDA, and percentage time active are in Table 1.

There was no correlation between MMR and meal size (% initial body mass) at 20°C (r=0.004, p=0.992) or at 30°C (r=0.064, p=0.860). There was also no correlation between SDA and meal size (% initial body mass) at 20°C (r=0.078, p=0.831) or at 30°C (r=0.216, p=0.549).

- *Comparison between starved metabolic rates and SMR at 20°C and 30 °C*

In eight individuals (n=4 at each temperature) we measured the starved metabolic rates and SMR of the same individual (Supplementary material: Table S3). For these paired samples considered in a separate analysis from the remaining individuals, there was no significant difference between the starved metabolic rates and SMR of larvae measured at 20°C (Z_2=1.826, p=0.068) or at 30°C (Z_2=1.461, p=0.144).

- *Temperature effects on gas exchange characteristics*

Out of the 20 larvae, 14 showed cyclic gas exchange (7 at 20°C and 7 at 30°C), four showed continuous gas exchange (1 at 20°C and 3 at 30°C) and two showed discontinuous gas exchange (both at 20°C) when at rest and post absorptive.

Since only two larvae showed discontinuous gas exchange no further analyses were done with the rate, volume and duration for the open (O-) and closed-flutter (CF-) phases (Supplementary material: Table S4). In larvae that showed cyclic gas exchange (Supplementary material: Table S5), there was no significant difference in burst rate, volume or duration between 20°C and 30°C. There was also no difference in interburst rate or volume between 20°C and 30°C. There was however a difference in interburst duration between 20°C and 30°C, with a significantly longer duration at 20°C (Table 1).

Table 1 Mean and standard deviation (SD) of key morphology and respirometry variables and the respective test statistic, degrees of freedom and p-values of comparisons made between 20°C and 30°C. Significant results are shown in bold. N refers to sample sizes.

Temperature	20°C (mean ± SD)	30°C (mean ± SD)	20°C vs. 30°C	N 20°C	N 30°C
Body mass (g) unfed	2.5422 ± 0.76	2.7199 ± 0.83	t_{21}=-0.5299, p=0.2071	10	13
Starved metabolic rate (mlCO_2/h/g)	0.0622 ± 0.02	0.0896 ± 0.07	Z_{21}=-0.6512, p=0.5149	10	13
Body mass (g) fed	2.4486 ± 0.97	2.1406 ± 1.31	t_{18}=0.5986, p=0.5569	10	10
Standard metabolic rate (mlCO_2/h/g)	0.0374 ± 0.03	0.0749 ± 0.04	**Z_{18}=-2.5324, p=0.0113**	10	10
Maximum metabolic rate (mlCO_2/h/g)	0.2434 ± 0.16	0.5894 ± 0.32	**Z_{18}=-3.0615, p=0.0022**	10	10
Active metabolic rate (mlCO_2/h/g)	0.2774 ± 0.24	0.4252 ± 0.15	t_5=0.9183, p=0.4006	4	3
Aerobic scope (mlCO_2/h/g)	0.2060 ± 0.14	0.5145 ± 0.32	**Z_{18}=-2.9103, p=0.0036**	10	10
Specific dynamic action (mlCO_2/g)	0.5180 ± 0.32	1.8607 ± 1.75	**Z_{18}=-3.2127, p=0.0013**	10	10
Activity time (%)	18.55 ± 2.63	25.55 ± 15.16	Z_{10}=-0.3248, p=0.7453	5	7
Burst rate (mlCO_2/h)	0.2586 ± 0.11	0.3006 ± 0.24	t_{12}=-0.4239, p=0.6792	7	7
Burst volume (mlCO_2)	0.0298 ± 0.01	0.0296 ± 0.03	t_{12}=0.0123, p=0.9904	7	7
Burst duration (s)	425 ± 152	335 ± 147	t_{12}=1.1283, p=0.2812	7	7
Interburst rate (mlCO_2/h)	0.0369 ± 0.03	0.0651 ± 0.05	t_{12}=-1.2508, p=0.2348	7	7
Interburst volume (mlCO_2)	0.0078 ± 0.005	0.0086 ± 0.007	t_{12}=-0.2300, p=0.8219	7	7
Interburst duration (s)	796 ± 341	449 ± 74	**t_{12}=2.6351, p=0.0218**	7	7

4. Discussion

We report here the results of respiratory gas exchange investigations of a diverse range of metabolic responses of a poorly studied Cerambycidae beetle larvae under laboratory conditions. Our study showed that *C. newmannii* larvae are tolerant to resource limitations compared to other insect larvae. For example, third instar larvae of *Dendrolimus pini* (Lepidoptera: Lasiocampidae), an economically important pest of *Pinus* spp. that has undergone a range expansion in Europe, survived a maximum of 28 days under starvation (Lukowski et al., 2020). There are however several examples of insect larvae that survive to a similar extent (>2 months) under starvation (see Rotkopf and Ovadia, 2014). The ability of *C. newmannii* to survive extended periods under stress and their relatively high aerobic scope at 30°C could have aided in their ability to invade a novel environment. Additionally, the present of discontinuous gas exchange in *C. newmannii* perhaps indicates a physiological capacity for metabolic suppression since discontinuous gas exchange is associated with low metabolic rates (Javal et al., 2019; Matthews and Terblanche, 2015) although we did not find much evidence for a strong metabolic suppression starvation strategy.

In insects and many invertebrate ectotherms, the most important short-term determinant of metabolic rate after accounting for body mass is ambient temperature (e.g. review in Chown and Nicolson, 2004; Hochachka and Somero, 2002; Nespolo et al., 2003). In this study, we found the classic Q10 effect of a significant, approximately two-fold, increase in SMR at 30°C over 20°C. Other factors that are known to influence metabolism include activity and feeding status, yet few studies disentangle these effects fully across multiple environmental conditions (Halsey et al., 2015). While feeding effects are widely reported in metabolic studies of insects, typically the time-courses of these responses are not well characterised, and in only a few cases is this undertaken at different temperatures (Hill et al., 2020; McCue et al., 2016a, 2016b). In this study, the average SDA of larvae measured at 20°C was significantly lower compared to larvae measured at 30°C. There are a few possible interpretations of this result. First, it could indicate that greater SDA at a warmer temperature could reduce the allocation of limited energy to other energy budget components (Boggs, 2009). Alternatively, it could reflect metabolic upregulation in the cold and suggest adaptations to facilitate quicker digestion and growth under cooler conditions (see discussion in Terblanche et al., 2010). We argue that an alternative interpretation could be that our results suggest that *C. newmannii* digest meals more efficiently – at least when expressed in

terms of energetic cost – at 20°C compared to 30°C. Further work is however required to disentangle the potential source(s) of this variation.

Accurately determining the cost of digestion (SDA) in *C. newmannii* was challenging for a number of reasons. Firstly, the larvae would not always readily feed in the laboratory unless they were living inside sugarcane stalks. However, since the larvae were embedded in the sugarcane, we could not determine the exact time at which an individual larva fed despite daily weighing. Therefore, we could not fully capture the precise start point, and hence, the entire time-course, of the postprandial metabolic response in this study. This is a challenge of determining SDA noted in other studies too (McCue et al., 2016b). Another constraint in calculating the cost of digestion (SDA) in *C. newmannii* was due to larvae remaining active during the digestion period. Another study showed that in active insects the increase in metabolism due to feeding was not apparent because it is smaller than the metabolic elevation due to activity (Nespolo et al., 2005). Therefore, it is possible that the effect of activity metabolism masked the effects of digestion costs on the metabolic rates in *C. newmannii* to some extent. These constraints can explain why our results showed that a larger meal size – across the range we measured – did not lead to a higher SDA (or MMR) at either temperature, contrary to many studies that have found an increase in meal size to be matched by a corresponding increase in SDA (reviewed in Secor, 2009). There are however other examples where the relative cost of SDA is apparently unrelated to meal size (e.g. in a Neotropical frog (Timpone et al., 2019)). Despite the higher cost of digestion (SDA) at 30°C, *C. newmannii* larvae also showed a higher aerobic scope (AS) at 30°C. Higher AS and SDA at higher ambient temperatures might also indicate a capacity to rapidly capitalize on scarce or energy poor resources by quickly digesting that food when its available. Furthermore, a higher AS has been argued to be a determinant of fitness-related parameters in invasive fish (Marras et al., 2015; Steell et al., 2019).

We found no difference in metabolic rates of starved larvae between 20°C and 30°C. We did however find a significant difference in the metabolic rates of resting, post-absorptive larvae (i.e. SMR) between 20°C and 30°C, suggesting that, under starved conditions, the effect of temperature on metabolic rate is not as evident. Not all larvae were starved for the same amount of time (ranged between 5 and 9 days) but the results showed no association between starved metabolic rates and duration of the starvation period at 20°C or at 30°C. Therefore, a longer period of starvation did not lead to a lower metabolic rate as expected based on previous starvation studies. For example, the adult hematophagous bed bug (*Cimex*

lectularius) showed a decline in mass-specific metabolic rate with period of starvation (DeVries et al., 2015). While it can be argued that taking fuel use (carbohydrate, lipid or protein stores) into account during metabolic rate estimation in starvation studies is essential (Sinclair et al., 2011), these effects are unlikely to explain the lack of variation we see in *C. newmannii*.

Depressing metabolic costs under stress (such as starvation) would decrease the amount of endogenous fuels an organism uses during this period and thus increase its capability to survive (Secor and Carey, 2016). In this study we did not find significant metabolic depression under starvation. It is possible that the starvation period larvae were subjected to was not long enough to induce a metabolic rate response. Many Cerambycidae larvae, such as *C. newmannii*, have a long developmental time (>2 years) and often feed on dead or decaying wood (Ferreira, 1980) which is an energy poor substrate (Walczynska, 2007, 2009). Since this type of food is poor in nutrients, insects that feed on it show several specific adaptations (Walczynska, 2007). Therefore, it is possible that *C. newmannii* larvae are well-adapted to tolerate starvation. This is supported by the survival assays that showed that larvae did not seem to be greatly affected by starvation over the period we assessed them (45 days). However, the sample size used for the comparison of metabolic rates under starved and standard conditions was perhaps relatively small and therefore results should be interpreted with caution. Nevertheless, having a starved metabolic rate that is relatively temperature insensitive, coupled to the ability to rapidly upregulate metabolic rate upon resource provisioning, could be a substantial advantage for coping in resource poor, seasonal or fluctuating environments.

Discontinuous gas exchange was observed in two larvae that were resting at 20°C. This supports the idea that discontinuous gas exchange can be expressed, albeit only during inactivity, possibly attributed to a transition from central to peripheral nervous system control of respiration (Thienel et al., 2015; Matthews and Terblanche, 2015). This emphasizes the importance of allowing adequate time, and accounting for behavior, when measuring insect gas exchange patterns. The only significant difference we detected in the gas exchange characteristics between 20°C and 30°C was in the interburst duration, which was significantly longer at 20°C. This suggests that the regulation of gas exchange is not regulated through differences in the burst periods in *C. newmannii*, but rather interburst periods, unlike many other beetles examined to date (e.g. Chown and Davis, 2003; but see also flies in Basson and Terblanche, 2011).

As an organism expands its geographic range, it is likely to encounter one or several abiotic and biotic stressors. If it is able to adapt or rapidly overcome these stressors, it may continue range expansion unrestricted and invade diverse, novel environments. For example, starvation stress can occur when insects suffer from prolonged food shortages due to changes in food quality or availability, or due to seasonality or food depletion (Wu et al., 2016). A higher aerobic scope leads to an increased capability of an organism to support processes such as digestion, locomotion and growth, especially so in the case of intermittent and unpredictable availability of high-quality food (Halsey et al., 2018). This will, in turn, enable the organism to potentially escape or avoid predators, or more readily search for food, which increases overall fitness and an organism's ability to overcome stressors in the new environment. *Cacosceles newmannii* may further be faced with stressors such as desiccation and/or starvation during a transition from one host plant to another and when sugarcane was ploughed out in an effort to stop the spread of *C. newmannii*. Furthermore, sugarcane grows rapidly and is harvested at maturity (6-9 months) and can thus be abundant or absent sporadically in the landscape. *Cacosceles newmannii* also feed on other host plants, for example, plants in the family Poaceae (Smit et al., in review) where water shortages could be a common occurrence. Clarifying the physiological mechanisms related to digestion, starvation, and metabolic traits could help in predicting which other host plants of *C. newmannii* could be viable hosts, or agricultural plants at risk of infestation, in the future. Consequently, we argue that our study contributes to understanding the species invasion of novel habitats for three primary reasons. First, it's clear that *C. newmannii* is capable of rapidly changing its niche, as evidenced by the sudden emergency as an outbreak pest on sugarcane - an environment distinctly different in several fundamental ways from its likely native host environment. Second, since the native range of the species covers several climatic zones with large thermal variability, understanding the temperature dependence of the fundamental physiological traits studied here is important in predicting survival and growth in different climate regions. Third, adult dispersal and fitness depend on the conditions the larvae face. A well-fed and optimally growing larva will be more likely to turn into a large adult with energy to spare for dispersal and reproduction; adults are non-feeding, so the energy budget of the adult is determined by the quality of the larva.

To conclude, this study provides novel data for developing predictions about metabolic rates of *C. newmannii* under ecologically-relevant temperatures in the field. From a pest management perspective, this information can be used to start to estimate the potential of *C.*

newmannii to spread to other novel environments and indirectly inform population dynamic or risk models (e.g. Malishev et al., 2017) or in habitat or species management planning (e.g. Tomlinson, 2019). Future studies determining the metabolic cost of digestion (SDA) of *C. newmannii* feeding on the native host plants, thought to be species in the family Fabaceae (Smit et al., in review), before the switch to sugarcane could prove a valuable point of comparison to the results of this study. Overall, we argue here that metabolic flexibility – in the broadest sense - may be a key adaptation that facilitates invasion of novel environments and allows populations to survive across a wide range of seasonal or pulsing resource fluctuations.

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References

Basson, C.H., Terblanche, J.S., 2011. Respiratory pattern transitions in three species of Glossina (Diptera, Glossinidae). Journal of Insect Physiology, 57, 433–443.

Boardman, L., Terblanche, J.S., 2015. Oxygen safety margins set thermal limits in an insect model system. The Journal of Experimental Biology, 218, 1677–1685.

Boggs, C.L., 2009. Understanding insect life histories and senescence through a resource allocation lens. Functional Ecology, 23, 27–37.

Carrara, R., Vazquez, D.P., 2010. The species energy theory: a role for energy variability. Ecography, 33, 942–948.

Chappell, M.A., Rogowitz, G.L., 2000. Mass, temperature and metabolic effects on discontinuous gas exchange cycles in Eucalyptus-boring beetles (Coleoptera: Cerambycidae). Journal of Experimental Biology, 203, 3809–3820.

Chown, S.L., Davis, A.L.V., 2003. Discontinuous gas exchange and the significance of respiratory water loss in scarabaeine beetles. Journal of Experimental Biology, 206, 3547–3556.

Chown, S.L., Nicolson, S.W., 2004. Insect Physiological Ecology: mechanisms and patterns. Oxford University Press, New York.

Chown, S.L., Marais, E., Picker, M.D., Terblanche, J.S., 2006. Gas exchange characteristics, metabolic rate and water loss of the Heelwalker, *Karoophasma biedouwensis* (Mantophasmatodea: Austrophasmatidae). Journal of Insect Physiology, 52, 442–449.

Claireaux, G., Lefrancois, C., 2007. Linking environmental variability and fish performance: integration through the concept of scope for activity. Philosophical Transactions of the Royal Society B: Biological Sciences, 362, 2031–2041.

Clark, T. D., Jeffries, K. M., Hinch, S. G. and Farrell, A. P., 2011. Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. Journal of Experimental Biology, 214, 3074–3081.

Contreras, H.L., Bradley, T.J., 2010. Transitions in insect respiratory patterns are controlled by changes in metabolic rate. Journal of Insect Physiology, 56, 522–528.

Desforges, J., Marques, G.M., Beumer, L.T., Chimienti, M., Blake, J., Rowell, J.E., Adamczewski, J., Schmidt, N.M., van Beest, F.M., 2019. Quantification of the full lifecycle bioenergetics of a large mammal in the high Arctic. Ecological Modelling, 401, 27–39.

DeVries, Z.C., Kells, S.A., Appel, A.G., 2015. Effects of Starvation and Molting on the Metabolic Rate of the Bed Bug (*Cimex lectularius* L.). Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches, 88, 53–65.

Duncan, F.D., Krasnov, B., Mcmaster, M., 2002. Novel case of a tenebrionid beetle using discontinuous gas exchange cycle when dehydrated. Physiological Entomology, 27, 79–83.

Ferreira, G.W.S. 1980. The Parandrinae and the Prioninae of Southern Africa (Cerambycidae, Coleoptera). Memoirs of the National museum Bloemfontein.

Gibbs, A.G., Fukuzato, F., Matzkin, L.M., 2003. Evolution of water conservation mechanisms in *Drosophila*. Journal of Experimental Biology, 206, 1183-1192.

675 Halsey, L.G., Matthews, P.G.D., Rezende, E.L., Chauvaud, L., Robson, A.A., 2015. The
676 interactions between temperature and activity levels in driving metabolic rate: theory, with
677 empirical validation from contrasting ectotherms. Oecologia, 177, 1117-1129.

678 Halsey, L.G., Killen, S.S., Clark, T.D., Norin, T., 2018. Exploring key issues of aerobic scope
679 interpretation in ectotherms: absolute versus factorial. Reviews in Fish Biology and Fisheries,
680 28, 405–415.

681 Hanks, L.M., 1999. Influence of the larval host plant on reproductive strategies of
682 cerambycid beetles. Annual Review of Entomology, 44, 483–505.

683 Hill, S.J., Silcocks, S., Andrew, N.R., 2020. Impacts of temperature on metabolic rates of
684 adult *Extatosoma tiaratum* reared on different hostplant species. Physiological Entomology,
685 45, 7–15.

686 Hochachka, P.W., Somero, G.N., 2002. Biochemical Adaptation: mechanism and process in
687 physiological evolution. Oxford University Press, New York.

688 Irlich, U.M., Terblanche, J.S., Blackburn, T.M., Chown, S.L., 2009. Insect Rate-Temperature
689 Relationships: Environmental Variation and the Metabolic Theory of Ecology. American
690 Naturalist, 174, 819–835.

691 Ivanovic, J., Dordevic, S., Ilijin, L., Jankovic-Tomanic, M., Nenadovic, V., 2002. Metabolic
692 response of cerambycid beetle (*Morimus funereus*) larvae to starvation and food quality.
693 Comparative Biochemistry and Physiology Part A, 132, 555–566.

694 Javal, M., Thomas, S., Barton, M.G., Gillespie, D., Conlong, D.E., Terblanche, J.S., 2018.
695 Understanding the recent invasion of *Cacosceles newmanii* (Coleoptera: Cerambycidae) into
696 sugarcane from a thermal perspective. SASTA Congress, Durban, South-Africa, 14-16th
697 August 2018.

698 Javal., M., Thomas, S., Lehmann, P., Barton, M.G., Conlong, D.E., Du Plessis, A.,
699 Terblanche, J.S., 2019. The Effect of Oxygen Limitation on a Xylophagous Insect's Heat
700 Tolerance Is Influenced by Life-Stage Through Variation in Aerobic Scope and Respiratory
701 Anatomy. Frontiers in Physiology, 10: 1426.

702 Karasov, W.H., Martinez del Rio, C., 2007. Physiological Ecology: How Animals Process
703 Energy, Nutrients, and Toxins. Princeton University Press, Princeton, United States.

Kleynhans, E., Barton, M.G., Conlong, D.E., Terblanche, J.S., 2018. Population dynamics of *Eldana saccharina* Walker (Lepidoptera: Pyralidae): application of a biophysical model to understand phenological variation in an agricultural pest. Bulletin of Entomological Research, 108, 283-294.

Lukowski, A., Adamczyk, D., Karolewski, P., 2020. Survival and Recovery of the Pine-Tree Lappet *Dendrolimus pini* When Subjected to Simulated Starvation. Insects, 11, 67.

Malishev, M., Bull, C.M., Kearney, M.R., 2017. An individual-based model of ectotherm movement integrating metabolic and microclimatic constraints. Methods in Ecology and Evolution, 9, 472-489.

Marais, E., Klok, C.J., Terblanche, J.S., Chown, S.L., 2005. Insect gas exchange patterns: a phylogenetic perspective. Journal of Experimental Biology, 208, 4507–4516.

Marras, S., Cucco, A., Antognarelli, F., Azzurro, E., Milazzo, M., Bariche, M., Butenschön, M., Kay, S., Di Bitetto, M., Quattrocchi, G., 2015. Predicting future thermal habitat suitability of competing native and invasive fish species: from metabolic scope to oceanographic modelling. Conservation physiology, 3, cou059

Marron, M.T., Markow, T.A., Kain, K.J., Gibbs, A.G., 2003. Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila*. Journal of Insect Physiology, 49, 261–270.

Marshall, D.J., McQuaid, C.D., 2011. Warming reduces metabolic rate in marine snails: adaptation to fluctuating high temperatures challenges the metabolic theory of ecology. Proceedings of the Royal Society B, 278, 281–288.

Matthews, P.G.D., Terblanche, J.S. 2015. Chapter One - Evolution of the Mechanisms Underlying Insect Respiratory Gas Exchange. Advances in Insect Physiology, 49,1–24.

Mattson, W.J., 1980. Herbivory in relation to plant nitrogen content. Annual Review of Ecology and Systematics, 11, 119–161.

Matzkin, L.M., Watts, T.D., Markow, T.A., 2009. Evolution of stress resistance in *Drosophila*: interspecific variation in tolerance to desiccation and starvation. Functional Ecology, 23, 521-527.

McCue, M.D., 2006. Specific dynamic action: A century of investigation. Comparative Biochemistry and Physiology, 144, 381–394.

McCue, M.D., 2012. Comparative Physiology of Fasting, Starvation, and Food Limitation. Springer, Berlin; New York.

McCue, M.D., Boardman, L., Clusella-Trullas, S., Kleynhans, E., Terblanche, J.S., 2016a. The speed and metabolic cost of digesting a blood meal depends on temperature in a major disease vector. Journal of Experimental Biology, 219, 1893–1902.

McCue, M.D., Salinas, I., Ramirez, G. and Wilder, S., 2016b. The postabsorptive and postprandial metabolic rates of praying mantises: Comparisons across species, body masses, and meal sizes. Journal of Insect Physiology, 93–94, 64–71.

McCue, M.D., Terblanche, J.S., Benoit, J.B., 2017. Learning to starve: impacts of food limitation beyond the stress period. Journal of Experimental Biology, 220, 4330–4338.

Miller, G.A., Clissold, F.J., Mayntz, D., Simpson, S.J., 2009. Speed over efficiency: locusts select body temperatures that favour growth rate over efficient nutrient utilization. Proceedings of the royal society B, 276, 3581–3589.

Munyiri, F.N., Ishikawa, Y., 2005. Feeding glucose or sucrose, but not trehalose, suppresses the starvation-induced premature pupation in the yellow-spotted longicorn beetle, *Psacothea hilaris*. Journal of Insect Physiology, 51, 1005–1012.

Nespolo, R.F., Lardies, M.A., Bozinovic, F., 2003. Intrapopulational variation in the standard metabolic rate of insects: repeatability, thermal dependence and sensitivity (Q10) of oxygen consumption in a cricket. Journal of Experimental Biology, 206, 4309–4315.

Nespolo, R.F., Castaneda, L.E., Roff, D.A., 2005. The effect of fasting on activity and resting metabolism in the sand cricket, *Gryllus firmus*: a multivariate approach. Journal of Insect Physiology, 51, 61–66.

Norin, T., Malte, H., 2011. Repeatability of standard metabolic rate, active metabolic rate and aerobic scope in young brown trout during a period of moderate food availability. Journal of Experimental Biology, 214, 1668–1675.

Pörtner, H. O., 2012. Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. Marine Ecology Progress Series, 470, 273–290

R Core Team (2018) R: A language and environment for statistical computing. The R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

764 Rosendale, A.J., Dunlevy, M.E., McCue, M.D., Benoit, J.B., 2019. Progressive behavioural,
765 physiological and transcriptomic shifts over the course of prolonged starvation in ticks.
766 Molecular Ecology, 28, 49–65.

767 Rotkopf, R., Ovadia, O., 2014. Examining growth rate and starvation endurance in pit-
768 building antlions from Mediterranean and desert regions. Ecological Entomology, 39, 94–
769 100.

770 SASRI (South African Sugarcane Research Institute), 2020.
771 <https://sasri.sasa.org.za/weatherweb_legacy/weatherweb.ww_menus.menu_frame?menuid=
772 1>

773 Secor, S.M., Faulkner, A.C., 2002. Effects of meal size, meal type, body temperature, and
774 body size on the specific dynamic action of the marine toad, *Bufo marinus*. Physiological and
775 Biochemical Zoology, 75, 557–571.

776 Secor, SM., 2009. Specific dynamic action: a review of the postprandial metabolic response.
777 Journal of Comparative Physiology B, 179,1–56.

778 Secor, S. M., Carey, C., 2016. Integrative physiology of fasting. Comprehensive Physiology,
779 6, 773–825.

780 Smit, C., Javal, M., Conlong, D.E., Hall, G., Terblanche, J.S., in review. Host range
781 determination in a novel outbreak pest of sugarcane, *Cacosceles newmannii* (Coleoptera:
782 Cerambycidae), inferred from stable isotopes.

783 Steell, SC, Van Leeuwen, TE, Brownscombe, Cooke SJ, Eliason, EJ., 2019. An appetite for
784 invasion: digestive physiology, thermal performance and food intake in lionfish (*Pterois*
785 spp.). Journal of Experimental Biology, 222, jeb209437.

786 Sinclair, B.J., Bretman, A., Tregenza, T., Tomkins, J.L., Hosken, D.J., 2011. Metabolic rate
787 does not decrease with starvation in *Gryllus bimaculatus* when changing fuel use is taken into
788 account. Physiological Entomology, 36, 84–89.

789 Storey, K.B., 1998. Survival under stress: molecular mechanisms of metabolic rate
790 depression in animals. South African Journal of Zoology, 33, 55-64.

791 Storey, K.B (ed)., 2004. Functional metabolism: Regulation and Adaptation. Wiley-Liss,
792 Inc., Hoboken, New Jersey.

Terblanche, J.S., Clusella-Trullas, S., Chown, S.L., 2010. Phenotypic plasticity of gas exchange pattern and water loss in *Scarabaeus spretus* (Coleoptera: Scarabaeidae): deconstructing the basis for metabolic rate variation. The Journal of Experimental Biology, 213, 2940–2949.

Terblanche, J.S., Woods, H.A., 2018. Why do models of insect respiratory patterns fail? Journal of Experimental Biology, 221, jeb130039.

Thienel, M., Canals, M., Bozinovic, F., Veloso, C., 2015. The effects of temperature on the gas exchange cycle in *Agathemera crassa*. Comparative Biochemistry and Physiology, Part A, 183, 126–130.

Timpone, L.T., Gavira, R.S.B., Andrade, D.V., 2019. Effects of temperature and meal size on the postprandial metabolic response of *Leptodactylus latrans* (Anura, Leptodactylidae). Journal of Experimental Zoology, 333, 79–87.

Tomlinson, S., 2019. The construction of small-scale, quasi-mechanistic spatial models of insect energetics in habitat restoration: A case study of beetles in Western Australia. Diversity and Distributions, 26, 1016-1033.

Walczynska, A., 2007. Energy budget of wood-feeding larvae of *Corymbia rubra* (Coleoptera: Cerambycidae). European Journal of Entomology, 104, 181–185.

Walczynska, A., 2009. Bioenergetic strategy of a xylem-feeder. Journal of Insect Physiology, 55, 1107–1117.

Wang, T., Zaar, M., Arvedsen, S., Vedel-Smith, C., Overgaard, J., 2003. Effects of temperature on the metabolic response to feeding in *Python molurus*. Comparative Biochemistry and Physiology- Part A, 133, 519–527.

Way, M.J., Conlong, D.E., Rutherford, R.S., Sweby, D.L., Gillespie, D.Y., Stramack, R.A., Lagerwall, G., Grobbelaar, E., Perissinotto, R., 2017. *Cacosceles* (Zelogenes) *newmannii* (Thomson) (Cerambycidae:Prioninae), a new pest in the South African sugarcane industry. Proceedings of the South African Sugar Technologist Association, 90, 62–65.

Wegener, G. ,1996. Flying insects: model systems in exercise physiology. Experientia, 52, 404–412.

Wright, D.H., 1983. Species-energy theory: An extension of species-area theory. Oikos, 41, 496–506.

Wu, H.-W., Li, X.-C., Liu, H.-X., 2016: Starvation resistance of invasive lace bug *Corythucha ciliate* (Hemiptera: Tingidae) in China. Entomologica Fennica, 27, 8–14.

Zang, D., Xiao, Z., Zeng, B., Li, K., Tang, Y., 2019. Insect behaviour and physiological adaptation mechanisms under starvation stress. Frontiers in Physiology, 10, 163.

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Highlights

- Thermal effects on metabolic parameters were estimated in a novel sugarcane pest
- *C. newmannii* tolerate resource limitations for at least 45 days
- Metabolic responses to temperature depend on food availability
- Food provision elicits metabolic-temperature responses

- Starvation dampens metabolic-temperature effects
- Metabolic flexibility may facilitate invasion of novel environments